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# QUANTITATIVE RELATIONS BETWEEN CHROMATIN AND CYTOPLASM IN THE GENUS ARCELLA, WITH THEIR RELATIONS TO EXTERNAL CHARACTERS

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A problem that has attracted considerable attention during the past twenty years is that of the quantitative relations between the nucleus and the cytoplasm of animal cells.

According to Richard Hertwig, who has been the foremost advocate of the nucleo-cytoplasmic-relation theory, a balance between nuclear and cytoplasmic masses exists in the normal cell, this balance being due to the interchange of materials between nucleus and cytoplasm. This state of equilibrium may be disturbed by such factors as changes in temperature, over-feeding or starvation. The result of these disturbing agents is an excess of nuclear over cytoplasmic materials. This excess of nuclear material leads to the depression of the cell, which finally ends in death unless normal mass relations are re-established in some way. The restoration of the normal equilibrium may be attained by the giving up of nuclear material to the cytoplasm, by ordinary cell division, or by the addition of a foreign element through conjugation. Hertwig and others have attempted to account for many of the complicated stages in the life cycles of Protozoa with this theory, but while this hypothesis is apparently applicable to many phenomena it will not bear close analysis, and a review of the extensive literature on this subject reveals a fatal lack of data on which to base the theory.

The material that I have used in my investigations consisted of several species of shelled fresh-water Protozoa of the genus Arcella. Arcella dentata has a shell with tooth-like projections extending out from the periphery. This shell averages about 130 microns in diameter and 50 microns in thickness.

There is a circular mouth opening in the ventral wall of the shell, through which protoplasmic extensions are pushed out that serve as locomotor organs and for capturing food. The cytoplasmic body within the shell contains two nuclei situated on opposite sides of the mouth opening. These nuclei are of the vesicular type, with the chromatin aggregated into a spherical mass in the center. Since the shell is almost transparent, especially in young specimens, it is easy to measure both the entire nucleus and the chromatin-mass within it, in the living animal. This makes Arcella peculiarly favorable for nucleo-cytoplasmic studies. The usual method of reproduction is binary division. When a certain amount of protoplasm has been built up within the shell and environmental conditions are favorable, the cytoplasm protrudes from the mouth of the shell and secretes a new shell; then, approximately one-half of the protoplasm passes back into the old shell, and the two shells separate. It is thus possible to distinguish between parent and offspring.

The experiments performed on pure lines of Arcella dentata prove that a definite relation exists between nuclear number and cell size within each pure line.

One of the members of line 150 was cut in two so that each half contained a single nucleus. Both halves continued to live and reproduce; the offspring were slightly irregular in shape, but the spines could be counted easily and the diameter measured. These offspring were smaller than the original parent and possessed fewer spines. They were each provided with only one nucleus. From these two uninucleate half-specimens were derived 209 uninucleate descendants which had a mean spine number of 11 and a mean diameter of 116 microns.

After a number of generations that differed in different cases, all of the uninucleate specimens produced empty shells and became binucleate again. Apparently during this process the single nucleus divided into two, as in ordinary division, but the new shell that was formed, was cast off empty, and all of the cytoplasm and both nuclei were retained in the parent shell. The binucleate specimens thus formed, gave rise at once to binucleate offspring. These offspring were larger than the parent; and their offspring were still larger. In this way a gradual increase in diameter and in spine number took place generation after generation, until at the end of the third or fourth generation, an equilibrium was established and a mean diameter and spine number were regained characteristic of the line before the experiments were begun. Thus in line 150, the size of the organisms and the characteristics of the shell depend upon the number of nuclei, and each nucleus is accompanied by a rather definite quantity of cytoplasm.

Other lines of Arcella dentata were subjected to this and other kinds of operations and the data obtained fully confirm the conclusion just stated.

Arcella polypora differs from Arcella dentata in the absence of spines and in the greater number of nuclei. In this species the nuclei are distributed at approximately equal distances from one another. It was found that within a line derived by fission from a single specimen, the number of nuclei varied. In line 5 they varied from 3 to 7. When the diameters of these specimens were compared with the number of nuclei they contained, a remarkably high correlation was revealed. The mean diameter of specimens with 3 nuclei was 109 microns; of those with 4 nuclei, 113; of those with five nuclei, 120; of those with six nuclei, 127; and of those with seven nuclei, 130 microns. It is evident that as the number of nuclei increases the size of the organism increases, and that in this species as in Arcella dentata, a rather definite amount of cytoplasm accompanies each nucleus. No specimens with less than 3 nuclei appeared in the cultures, so operations were resorted to, in order to obtain individuals with one and two nuclei. Specimens were cut into pieces, and these pieces continued to live and reproduce. From these pieces a few offspring with one and 2 nuclei were obtained. Specimens with one nucleus averaged 82 microns in diameter; and those with two nuclei, 86 microns. Here, as in normal specimens, size and nuclear number are closely correlated.

Other lines of Arcella polypora behaved in similar fashion, but the data show that the ratio between nuclear number and cell size differs markedly in the different lines. Thus the number of nuclei in line 34 ranged from 5 to 10, but the specimens were smaller than were those with a lesser number of nuclei in line 5. The specimens in line 5 with 4 nuclei were similar in size to those in line 34 with 8 nuclei; those in line 5 with 5 nuclei were about as large as those in line 34 with 9 nuclei; and those in line 5 with 6 nuclei, approximated in diameter those in line 34 with 10 nuclei.

This condition suggested the possibility that size in these organisms is controlled not by the number of nuclei, but by the *amount of chromatin* within the nuclei. Accordingly, measurements were made of the chromatin masses in specimen from a number of lines, and this hypothesis was confirmed in a remarkable manner.

Measurements were first obtained of specimens of Arcella dentata. It soon became evident that in this species, the larger the specimen the greater the amount of chromatin within the nuclei. A chromatin-cytoplasmic mass relation was thus established. Measurements were then made of the chromatin masses in specimens from lines 5 and 34 of Arcella polypora. From these measurements, the volumes of the chromatin masses were computed, and they were found to be greater in line 5 than in line 34. For example, the total volume of the 6 chromatin masses in an average specimen of line 5 proved to be approximately equal to the total volume of the 10 chromatin masses of an average specimen of the same size in line 34. Similar conditions were found to exist when the total volume of chromatin within other specimens of line 5 was compared with the total volume of chromatin within specimens of the same size in line 34. These data prove that the quantity of chromatin contained in specimens of the same size within the two lines, 5 and 34, was very nearly the same, regardless of the variations in the number of nuclei. Thus, in Arcella polypora, as in Arcella dentata, there is a certain amount of chromatin associated with a certain amount of cytoplasm, and a definite chromatin-cytoplasmic-mass-relation is shown to exist.

The data presented above not only show a mass relation between chromatin and cytoplasm, but also a relation between chromatin mass and external measurable characters. For example, in Arcella dentata, both the diameter of the shell and the number of spines are correlated with the chromatin mass; and in Arcella polypora, the diameter of the shell also depends upon the total volume of chromatin.

A summary of some selection work that I carried on last year with Arcella dentata was published in the October number of the PROCEEDINGS of this

Academy. I concluded from this work on selection, that from the descendants of a single specimen produced by binary division, lines could be distinguished that were hereditarily diverse as regards spine number and diameter. The present study indicates that these heritable diversities may have been due to changes in the volume of the chromatin. These chromatin-cytoplasmic studies also have a bearing on the selection work carried on by various investigators, notably by Jennings with Difflugia and by Root with Centropyxis. Arcella, Difflugia, and Centropyxis all belong to the same group of Protozoa, the fresh-water Rhizopods; but the nuclei can not be seen in either Difflugia or Centropyxis, and hence at least some of the results obtained by Jennings and by Root may have been due to changes in nuclear number and consequently in chromatin mass, rather than an hereditary change in the chromatin itself as seemed probable. An increase or decrease in nuclear number, however, does not account for simultaneous and independent diversities such as Jennings found in Difflugia with respect to shell diameter and length of spines, unless the assumption is made that certain nuclei exert an influence upon certain shell characters and other nuclei upon other shell characters. Evidence was obtained from my studies of Arcella polypora that hereditarily diverse strains with respect to nuclear number and shell diameter could be distinguished within a single line. More data regarding this and other related problems are very desirable.

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# *A THEOREM ON POWER SERIES, WITH AN APPLICATION TO CONFORMAL MAPPING*

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*Theorem: Given a power series* $w(z) = \sum_0^\infty a_n z^n$ *convergent for* $|z| < 1$ *and such that* $\sum_0^\infty n\,|a_n|^2$ *converges, and writing*

$$z = 1 - \rho e^{\theta i}, \; z' = 1 - \rho e^{\theta' i},$$

*where* $\rho > 0$ *and* $\theta$ *and* $\theta'$ *vary with* $\rho$ *subject only to the conditions*

$$-\frac{\pi}{2} + \epsilon \leqq \theta \leqq \frac{\pi}{2} - \epsilon, \quad -\frac{\pi}{2} + \epsilon \leqq \theta' \leqq \frac{\pi}{2} - \epsilon,$$

$\epsilon$ *being positive but arbitrarily small, then*

$$w(z) - w(z') \to 0 \text{ as } \rho \to 0$$

*uniformly in respect to* $\theta$ *and* $\theta'$.